## APPENDIX A CLAIMS Serial No.: 10/583,179 Docket No.: 490352-3004/US

## **CLAIMS:**

- 1 49. (cancelled)
- 50. (New) A protein separation device comprising GroEL immobilised on a substrate, wherein the specificity of GroEL is directed to a particular protein, wherein GroEL is engineered by site-directed mutagenesis to have the substitutions, leucine 200 to arginine; serine 201 to glycine, and proline 202 to aspartate.
- 51. (New) The protein separation device as claimed in claim 50, in which the subtitutions introduce an integrin binding motif into a protein binding domain of GroEL.
- 52. (New) The protein separation device as claimed in claim 50, in which GroEL comprises a back-to-back double ring configuration.
- 53. (New) The protein separation device as claimed in claim 50, in which GroEL is in operative association with a co-factor.
- 54. (New) The protein separation device as claimed in claim 53, in which the co-factor is GroES.
- 55. (New) The protein separation device as claimed in claim 50, in which the chaperone is obtainable from a microbial source selected from the group consisting of *Escherichia spp.*, *Thermus spp. Streptococcus spp.*, *Staphylococcus spp.*, *Bacillus spp.*, *Leptospira spp.*, *Spirillum spp.*, *Lactobacillus spp.*, *Mycoplasma spp.*, *Pseudomonas spp.*, *Streptomyces spp.*, *Corynebacterium spp.*, *Bacteroides spp.* and *Clostridium spp.*
- 56. (New) The protein separation device as claimed in claim 55, in which the *Escherichia spp*. microbial source is *Escherichia coli*.
- 57. (New) The protein separation device as claimed in claim 50, in which the substrate is a solid support of the array or bead type.

- 58. (New) The protein separation device as claimed in claim 50, in which the substrate is manufactured from a plastics material.
- 59. (New) The protein separation device as claimed in claim 50, in which the support of the array type is provided with a surface for immobilisation of a protein of the chaperone type thereon.
- 60. (New) The protein separation device as claimed in claim 59, in which the surface is comprised of moieties selected from the group consisting of nitriloacetic acid, avidin, streptavidin, carboxylates, quaternary amines, silicates, carbonyl diimidazoles and epoxides.
- 61. (New) The protein separation device as claimed in claim 59, in which the surface is provided with an hydrophobic barrier coating.
- 62. (New) The protein separation device as claimed in claim 50, in which said protein is separated from a biological sample selected from the group consisting of cerebrospinal fluid, urine and nipple aspirant.
- 63. (New) The protein separation device as claimed in claim 50, in which said protein is separated from a biological fluid or extract.
- 64. (New) The protein separation device as claimed in claim 1, in which said protein is separated from a biological sample which has been denatured.
- 65. (New) A protein separation device comprising GroEL immobilised on a substrate, wherein the specificity of GroEL is changed to a protein specificity of another chaperone protein, in which GroEL is engineered by site-directed mutagenesis to have the substitutions, Tyrosine 199 to Isoleucine; Tyrosine 204 to Isoleucine; Leucine 234 to Isoleucine; Leucine 237 to Isoleucine; Leucine 259 to Phenylalanine; Valine 263 to Leucine and Valine 264 to Phenylalanine.
- 66. (New) The protein separation device as claimed in claim 65, in which the substitutions replace the substrate binding specificity of GroEL, a group I chaperone, with that of thermosome, a group II chaperone.

- 67. (New) The protein separation device as claimed in claim 65, in which GroEL comprises a back-to-back double ring configuration.
- 68. (New) The protein separation device as claimed in claim 65, in which GroEL is in operative association with a co-factor.
- 69. (New) The protein separation device as claimed in claim 68, in which the co-factor is GroES.
- 70. (New) The protein separation device as claimed in claim 65, in which the chaperone is obtainable from a microbial source selected from the group consisting of *Escherichia spp.*, *Thermus spp. Streptococcus spp.*, *Staphylococcus spp.*, *Bacillus spp.*, *Leptospira spp.*, *Spirillum spp.*, *Lactobacillus spp.*, *Mycoplasma spp.*, *Pseudomonas spp.*, *Streptomyces spp.*, *Corynebacterium spp.*, *Bacteroides spp.* and *Clostridium spp.*
- 71. (New) The protein separation device as claimed in claim 70, in which the *Escherichia spp.* microbial source is *Escherichia coli*.
- 72. (New) The protein separation device as claimed in claim 65, in which the substrate is a solid support of the array or bead type.
- 73. (New) The protein separation device as claimed in claim 72, in which the substrate is manufactured from a plastics material.
- 74. (New) The protein separation device as claimed in claim 65, in which the support of the array type is provided with a surface for immobilisation of a protein of the chaperone type thereon.
- 75. (New) The protein separation device as claimed in claim 74, in which the surface is comprised of moieties selected from the group consisting of nitriloacetic acid, avidin, streptavidin, carboxylates, quaternary amines, silicates, carbonyl diimidazoles and epoxides.
- 76. (New) The protein separation device as claimed in claim 74, in which the surface is provided with an hydrophobic barrier coating.

- 77. (New) The protein separation device as claimed in claim 65, in which said protein is separated from a biological sample selected from the group consisting of cerebrospinal fluid, urine and nipple aspirant.
- 78. (New) The protein separation device as claimed in claim 65, in which said protein is separated from a biological fluid or extract.
- 79. (New) The protein separation device as claimed in claim 65 in which said protein is separated from a biological sample which has been denatured.